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Pesticide Resistance: Assessment of Risk and the Development and Implementation of Effective Management Strategies†

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Abstract: Insecticides, fungicides and herbicides are critical to successful crop production, but the development of pesticide resistance is a continual threat, especially to many of today's selective toxophores with specific binding sites. In order to manage resistance effectively, an assessment of genetic, ecological and operational risk factors is required, which must then be translated into meaningful local strategies that can be implemented through appropriate labelling of products and education of end users. Assessing resistance risk is a fundamental part of the development process for new molecules and is increasingly becoming a requirement of registration alongside toxicological and environmental risk data. Laboratory studies, including elucidation of target sites and metabolic degradation pathways, mutagenesis, computer models and cross-resistance tests, and field studies, including establishment of baseline sensitivities and evaluation of anti-resistance strategies, all play a part in such assessment. The challenge is then to devise management strategies which are relevant to local practice and actually reduce selection pressure to a point where product life is preserved. A preventative strategy should be in place at time of launch and for most pesticides, regional co-operation between all interested parties, of the kind advocated by the Resistance Action Committees of GCPF (Global Crop Protection Federation), increases the chance of success. Implementation of strategies *via* a universal product labelling system, already practised in some herbicide markets, is seen as a key way to improve product usage patterns. Monitoring resistance levels in field populations after product launch enables any fine tuning of tactics over time, for example in response to new technologies such as transgenic varieties being introduced. The limited successes in resistance management in Australia, Zimbabwe, Europe and USA are not so easily achieved in small-holder farming in developing countries, as exemplified by continuing problems in parts of India and China. Emphasis must be given to the education of growers and dealers in IRM and IPM, and improved extension services, in order to bring about a more sustainable approach to crop protection. © 1998 Society of Chemical Industry

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1 BACKGROUND

Successful production of food and fibre globally is dependent on the effective control of weeds, diseases, insects, mites and nematodes which reduce crop yields, hinder harvest operations and contaminate produce. Damage is reduced markedly through the judicious use of a range of agronomic, cultural and physical techniques, but the most valuable tool over the last few decades has been effective and reliable chemical toxophores. In fact, in 1997, global sales of pesticides amounted to \$30·2 billion.[1]

The continued success of pesticides is threatened by the evolution of resistance to key classes of herbicides, fungicides and insecticides. For example, cases of resistance have been confirmed for major toxophore types such as the herbicidal acetolactate synthase (ALS) inhibitors and acetyl-coenzyme A carboxylase (ACCase) inhibitors, the fungicidal sterol biosynthesis inhibitors, and the insecticidal pyrethroids. Concern has also been expressed about the threat of resistance developing to the narrow range of Bt (*Bacillus thuringiensis* Berl.) toxins currently expressed in transgenic crop varieties.[2] Such resistance usually leads to reduced efficacy but, depending on its frequency in a population, it does not necessarily equate with failure to achieve economic levels of control. However, when product failure does arise, the position is sometimes complicated by the fact that distinct chemical groups can have a common mode of action or similar metabolic degradation pathways, such as the ALS inhibitors (e.g. sulfonylurea, imidazolinone and triazolopyrimidine herbicides) and acetylcholinesterase inhibitors (OP and carbamate insecticides), leading to the phenomenon of cross-resistance.

To counter the problem of cross-resistance, the Herbicide Resistance Action Committee (HRAC), Fungicide Resistance Action Committee (FRAC), and Insecticide Resistance Action Committee (IRAC) have made good progress in attempting to introduce a single mode-of-action classification which recognises the potential for cross-resistance and is accepted globally (References 3, 4; Irving, S. R., 1997 pers. comm. and Brent, K., 1998 pers. comm.) and which will assist advisors and growers in implementing resistance-management practices based on sound scientific principles.

The objective of this paper is to examine the assessment of resistance risk in novel and established products and the development and communication of effective management strategies, two aspects of resistance crucially important to the agricultural industry. The focus will be primarily on resistance to insecticides and fungicides, as herbicide resistance has been reviewed recently by Powles *et al.*[5]

2 ASSESSING THE RISK

When presenting novel pesticides for registration, for example in the EU and USA, agrochemical companies will be required to present information pertaining to the likelihood of resistance developing to the new agent and to propose anti-resistance strategies to minimise such development.[6] This may seem relatively straightforward given the extensive knowledge on resistance, but in reality the task is both complex and difficult to achieve satisfactorily.

Resistance risk assessment is now scheduled on the development timetable of a new active ingredient in much the same way as other mandatory requirements (toxicology and environmental assessments, for example). The accepted series of studies, however, is necessarily less prescriptive.

Resistance risk assessment projects require information to be generated from a number of sources. Useful information can be gleaned as to the inherent resistance risk presented by the pesticide from biochemical mode-of-action studies, cross-resistance tests, mutagenesis and selection experiments (in the laboratory and/or the field) genetic studies and from analysing the variability in sensitivity of target populations prior to commercialisation. None of these studies is likely to provide a definitive answer to risk assessment. However, careful consideration of information from each of these areas and the likely use pattern of the pesticide allows the categorisation of the inherent resistance risks of different targets to the pesticide and permits the development of appropriate anti-resistance measures.

Information is presented below for insecticides and fungicides to demonstrate how research aids decision making and, with the benefit of hindsight, some of the limitations and exceptions that have arisen with previous case histories.

2.1 Mode of action

Lack of cross-resistance to existing pesticides is a prerequisite for the further development of a new insecticide, acaricide or fungicide. A first tactic to meet this requirement is to screen chemically novel molecules, which, by their very nature are most likely to lead to the discovery of toxophores active through a novel mode of action which is not subject to altered site resistances already present in pest populations.

Novel mode of action is determined by a failure to account adequately for the activity in the battery of in-vitro biochemical screens currently available for this purpose and by observations in in-vivo bioassays. A truly novel mode of action may take some years to elucidate fully as in the case of the recently introduced spinosad from Dow AgroSciences (Dutton, R., 1997, pers. comm.). However, elucidation of a novel mode of

action can make less contribution to assessing the resistance risk than if the molecule possesses a known mode of action, for, until an understanding of the potential for variation at that site of action is obtained, it is not possible to predict the risk (if any) of resistance developing through active site alteration. It may be considered that a specific mode of action may imply a higher risk of resistance developing than a less specific or multi-site action.[7] However, exceptions to this general rule exist. For example, the morpholine fungicides are specific inhibitors of sterol biosynthesis, yet they have been used for 30 years with little evidence of field resistance developing.[8] Conversely, resistance is documented in plant pathogens to the multi-site compounds, organomercury[9] and organotin.[10]

Novel compounds found to act at an 'established' target site are not necessarily rejected for further development, as they may act through binding at a distinct domain. For example, the *N*-alkyl amide insecticides worked on by Wellcome, BTG and others for many years[11,12] act at the sodium channel, but at a binding site distinct from that of the pyrethroids and DDT.[13] In addition to chemical novelty, these compounds showed the attractive property of negative cross-resistance in insects with *kdr* or *super-kdr* resistance, an alteration at the level of the sodium channel which confers resistance to pyrethroids. Unfortunately, this chemistry proved highly susceptible to the metabolic resistance mechanisms present in a wide range of potential target organisms,[14,15] and no examples have been commercialised to date.

2.2 Cross-resistance

A retrospective look at the prediction of resistance risk in insects and fungi suggests that it is greatly dependent on the chemical class (defined by the mode of action). In general, compounds within chemical classes exhibit similar resistance risk properties, as evidenced by the methyl benzimidazole carbamate (MBC), dicarboximide, phenylamide, sterol C-14 demethylation (DMI) inhibitors and morpholine fungicides. In these cases, this probably reflects similar mechanisms of resistance developing within classes based around changes to the target site. Similar trends are found amongst insects to the organophosphate (OP) pyrethroid and cyclodiene insecticides, but the link between the resistance mechanism and mode of action or target site seems particularly strong in fungi. This may be an accidental coincidence of the target sites involved to date or perhaps, as proposed by Hollomon *et al.*,[16] plant pathogenic fungi lack the non-specific detoxification and excretory systems developed by insects and mites which can confer cross-resistance to chemically unrelated compounds.

Lack of cross-resistance to existing (or even superseded) chemistry is determined by in-vitro and in-vivo biochemical and biological assays conducted using preparations from insects or pathogens from well-characterised resistant strains, including field strains. Such studies can be used as an essential component of the development of novel fungicides and insecticides in order to confirm that existing resistance phenotypes present in the population are well controlled by the new compound. Tests are designed to demonstrate that the response of a strain to a new compound is unrelated to the resistance profile of the strain to existing products. By this means, recent insecticide and fungicide introductions such as the chloronicotinyls[17] and strobilurins[18] have been shown to be in novel cross-resistance groups and thus represent excellent examples to aid the design of future anti-resistance programmes, but elucidate little about the inherent risk of resistance development.

Failure to demonstrate cross-resistance in biochemical assays, in strains of insect from the field or with known resistance mechanisms cannot provide any certainty of a long period of trouble-free sales before resistance develops. This concern is greatest for compounds with a relatively narrow spectrum of activity, such as the selective aphicide, pirimicarb. This compound offered respite to glasshouse ornamental growers facing severe aphid control problems due to resistance to OP products when it was introduced in the early 1970s. However, within three years of introduction in the UK, an unpredicted compound-specific resistance developed in *Aphis gossypii* Glover, conferred by an alteration at the active site.[19] This severely compromised the utility of the product in this particular outlet.[20]

This outcome potentially awaits any novel molecule directed at an insect or mite species with a proven history of pesticide resistance, and this concern is even greater when the compound is not the first of the chemical series. The consequences of resistance for companies introducing further neo-nicotinoid compounds, such as acetamiprid and thiamethoxam, are much more serious than to the company which introduced the series to the market several years earlier. Launching a new product into an environment in which there is cross-resistance to existing compounds could jeopardise the chances of achieving adequate return on the investment required to introduce the product. Further, these companies have a markedly more difficult task in assessing these risks, particularly as resistance to chloronicotinyl compounds has been confirmed in only a very few locations.[21] With isolated and localised reports of often low-level resistance, it is difficult to collate sufficient reliable data to commence evaluation of the factors which may influence the speed at which resistance may develop into a more widespread phenomenon. The hope is, of course, that any new compound will be one of the exceptions such as the acaricide, propargite, which remains largely unaffected by resistance after over 20 years' use against

targets prone to develop resistance rapidly (Graham, J., 1997, pers. comm.).

Indications of cross-resistance in early tests immediately provide uncertainty for the future of molecules in question. The combined skills of the biologists and business analysts are then required to interpret both biochemical and biological data, in conjunction with financial models, to assess the viability of the potential product in the light of the major financial investment required for the further development of the product. The dilemma facing such a group can be exemplified in the comparative development histories of two novel chemistries sharing the mode of action of gamma-aminobutyric acid (GABA) antagonism, namely Zeneca's aryl pyrimidinones[22] and the Rhône Poulenc phenyl pyrazoles, exemplified by fipronil.[23] Compounds acting in this manner may be resisted by strains possessing an alteration at the cyclodiene/lindane/picrotoxinin binding site on the GABA receptor, selected for by the widespread use of cyclodiene compounds in the 1960s. This was demonstrated to occur for both series of compounds (Reference 23; Dunbar, S. J., 1995, pers. comm.). Although the use of cyclodienes has decreased markedly owing to resistance and environmental concerns, and there is evidence of resistance instability in certain species,[24] the risk of rapid reselection has remained in some outlets.[25] An extensive review of the distribution and likely consequences of this resistance suggested that this risk was too great for the aryl pyrimidones, which had an insecticidal spectrum largely limited to dipteran and orthopteran public and human health pests. Cross-resistance to cyclodienes in resistant *Musca domestica* L., *Blatella germanica* L., *Anopheles gambiae* Giles and *Aedes aegypti* L. implied that over three-quarters of the commercial opportunities were potentially at risk from resistance, and the decision was subsequently taken not to develop these highly active compounds further. Although these risks exist for fipronil, the level of resistance is typically lower and the cross-resistance spectrum appeared less complete than with the aryl pyrimidinones. This factor, combined with a broader insecticidal spectrum, allowed development into agricultural, turf, public and animal-health outlets.

One further case of cross-resistance merits discussion: the phenomenon of negative cross-resistance where strains of a pest or pathogen resistant to one pesticide are more sensitive than the wild type to another pesticide. There are relatively few well-documented cases of this phenomenon for insect and acarid pests[14,26,27] yet negative cross-resistance seems to be a relatively common occurrence in fungi, for example, between different carboxanilides in field mutants of *Ustilago nuda* (Jensen) Rostrup,[28] and between benzimidazoles and diphenylamines in *Penicillium expansum* Link[29] and even between different DMI fungicides.[30] Negative cross-resistance between phenylcarbamates and benzimidazoles in grey mould, *Botryotinia fuckeliana* (de Bary) Whetzel (*Botrytis cinerea* Pers.),[31] formed the basis for the launch by Zeneca of 'Sumico', a mixture of diethofencarb (a phenylcarbamate) and methyl benzimidazole carbamate MBC, (a benzimidazole) on grapevines in France in the late 1980s. Strains have evolved which are resistant to both components of the mixture, which have unfortunately limited the success of the strategy. So far, the agrochemical industry has failed to exploit negative cross-resistance relationships to achieve stable anti-resistance strategies in the fields of fungal and insect control, but this possibility should not be overlooked for any new class of fungicide or insecticide, particularly where there are indications that the target site may present multiple binding domains and can tolerate many genetic changes whilst remaining functional.

2.3 Resistant mutants

One of the facets of risk assessment is the isolation and characterisation of resistant mutants either in model organisms or target pathogens. It is useful, with the aid of hindsight, to examine how such studies might have helped assess risk to major fungicide discoveries.

Resistance to the benzimidazoles developed in only a few years in pathogens such as *Sphaerotheca fuliginea* (Schlecht.) Pollacci and *B. cinerea.* This proclivity to resistance development was indicated by van Tuyl[32] who isolated benomyl-resistant mutants in several fungal species. This was confirmed in studies showing that resistance was usually controlled by a single gene[33,34] and that resistant strains were often as virulent as wild-type.[35] However, even in this apparently straightforward case it would have been difficult to predict that of the four regions of the β-tubulin gene that can mutate to give benzimidazole resistance in the laboratory, only mutants from one region, 196–200, are found in the field.[16]

A similar divergence between laboratory mutants and field mutants has been seen with resistance to the dicarboximides in *B. cinerea.* In both laboratory and field mutants, resistance is determined by a single polymorphic major gene[34] but the laboratory mutant phenotype (genotype *Daf1HR*) with high resistance factors, lower fitness (osmotic sensitivity) and cross-resistance to the unrelated phenylpyrrole fungicides has never been detected in the field.

With the phenylamide fungicides, data were available during early commercialisation showing that pathogenic, resistant mutants could be isolated in *Phytophthora megasperma* f.sp. *medicaginis* Dreschler following mutagenic treatment with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.[36] Contradictory data indicated that strains of *Phytophthora infestans* (Mont.) de Bary isolated *in vitro* had only low pathogenicity[37] and were taken to indicate a relatively low risk for this class. Had information been available about the single gene control of metalaxyl resistance in *Bremia lactucae*

Regel,[38] conclusions might have been more cautious, allowing strategies to be implemented to delay the rapid onset of resistance that occurred in European populations of late blight.

Finally, the sterol biosynthesis inhibitors (SBI) make an interesting case for consideration. Resistance to the DMIs has developed gradually over the last 15 years in many pathogens of cereal, fruit and vegetable crops.[39] However, changes have been characterised by quantitative shifts in sensitivity and DMIs are still used effectively in most of these crops. Could this have been predicted? Polygenic control of resistance was indicated in *Aspergillus nidulans* (Eidam) Winter[40] and *Nectria haematococca* Berk. & Broome *var. cucurbitae* (*Fusarium solani* (Mart.)).[41] Resistant strains of *Cladosporium cucumericum* Ell. & Arthur all had a lower fitness, prompting the authors to predict that resistance would not become a problem.[42] A similar model of polygenic resistance was proposed for field strains of *Erysiphe graminis* DC f.sp. *hordei* Marchal.[8,43] All of the above might have allowed accurate risk predictions to be made. However, it seems unlikely that it could have been predicted that resistance to another class of SBIs, the morpholines, would make no practical impact over the course of 20 years, given that a single gene in *E. graminis* f.sp. *hordei* controls resistance to fenpropimorph (and fenpropidin)[44] and that single-gene mutants resistant to fenpropimorph are easily isolated in *N. haematococca var.cucurbitae*.[45] To complicate the position further, Brown *et al.*[44] also support the position that DMI resistance in *E. graminis* f.sp. *hordei* is under major gene control.

Within insecticide development, the role of resistant mutants has primarily been restricted to the use of well-characterised strains for cross-resistance determination during the screening process. Susceptibility to metabolism can largely be predicted or determined for a novel molecule in a variety of ways, as mentioned in Section 2.2. Thus the greatest benefit of mutant insect strains would be in the assessment of risk due to alterations at the active site. However, the difficulty in assimilating sufficiently diverse genetic material and the subsequent operational factors involved in managing and rearing sufficient biological material for assay, has largely precluded this approach. As such, there are no documented cases of this technique being used to assess the risk of resistance developing to insecticides with a novel mode of action.

Extending the technique by selection preceded by exposure to a chemical or radiation mutagen offers some potential for fecund insects or mites with short generation times, although similar constraints apply. At Zeneca, strains of *Myzus persicae* Sulz. strongly resistant to imidacloprid were generated following exposure of a (theoretically) genetically diverse culture to DMS and subsequent selection with imidacloprid. Unfortunately, the extreme lack of fitness associated with these strains suggested that they bore no resemblance to anything likely to be found in the field. The technique is currently being developed further, although the question of how representative such mutants are, will always be raised. The recent progress with genetic mapping techniques suggests that, provided a viable resistance mechanism to a novel compound can be confirmed and the responsible loci determined, screening of field populations could be undertaken to determine the extent of, and frequency of, alleles conferring resistance in order to conduct genetic risk assessment.

In conclusion, the study of resistant mutants can provide guides to risk assessment but conclusions may vary significantly, dependent on the species chosen for study. With both insects and fungi, data can only be interpreted confidently with the benefit of hindsight. Perhaps as mutants are generated in an increasingly rational way, using PCR mutagenesis techniques to delete, insert or substitute defined segments of DNA in the target pest and pathogens so that the field resistance mechanisms can be studied, predictive ability will improve.

2.4 Baseline sensitivities and field strategy experiments

Determination of the range of sensitivities present in important pathogen populations prior to commercialisation of novel fungicides is now a routine part of their development. Similar practices are also undertaken increasingly with insecticides, particularly those with narrow spectra of activity or activity concentrated against pests with a history of resistance problems.

A broad range of baseline sensitivities may indicate the potential for resistance and would certainly stimulate further investigation,[46] although the use of baselines as a tool to assess the risk of resistance development is, in general, limited. The design of monitoring methodology can, however, strongly depend on baseline data. A pathogen with a very narrow range of baseline sensitivities, for example, *Plasmopara viticola* (Berk. & Curtis ex de Bary) Berl. & De Toni to strobilurins,[47] might be effectively monitored in the future using only one or two discriminating doses, whereas a broad baseline, for example, *Erysiphe graminis* f.sp. *hordei* to quinoxyfen[48] might indicate the need to follow a quantitative shift in sensitivities necessitating the use of many more doses.

In a number of cases, field strategy experiments have yielded useful risk indicators. In the 1980s, ICI (Zeneca) detected combined resistance to MBC and diethofencarb in *B. cinerea* taken from trial plots in the Loire valley prior to the launch of this mixture. Similarly, resistance to the anilinopyrimidines was detected again in *B. cinerea* at a Swiss trial site.[49] In both cases anti-resistance measures were strengthened early in the product lifespan by reducing the number of recommended sprays per season and developing mixtures. It

remains a contentious point whether such studies should be expanded and artificially high selection pressures created in the field in an attempt to obtain field resistant isolates. Perhaps the best approach is to use efficacy trials carried out as part of the development process to provide field populations for resistance analysis. Intensive sampling of such sites might, at least, reveal potentially high-risk situations prior to commercialisation. To provoke resistance in any other way in the field seems irresponsible.

Assessing the resistance risk associated with a pesticide is still very much an imprecise activity, like weather forecasting; a worthwhile but uncertain subject. In order to develop successful management strategies, the resistance risk element associated with the pest must also be taken into account. Fecund, easily dispersed pests with short generation times and the ability to generate variation through sexual recombination have an excellent track record at developing resistance. These and other factors such as the relative isolation of the target population are listed in the proposed resistance risk scheme by Rotteveel *et al.*[7] and must be taken into account when assessing the overall risk of resistance developing. Such pests demand more stringent strategies to reduce selection pressure and the overall risk of resistance developing.

3 DEVELOPMENT OF EFFECTIVE MANAGEMENT STRATEGIES

If chemically novel inhibitors have a mode of action similar to that of existing fungicides, cross-resistance studies may require more careful thought opposite the development of an acceptable management strategy. A general cross-resistance exists between the DMI group of SBIs, but the correlation of resistance between individual pairs of DMIs varies considerably both within and between species of temperate cereal pathogens.[50–53] Furthermore, cross-resistance relationships for SBIs may be different from one population of *Pyrenophora teres* Dreschler to another,[52] and, in *Mycosphaerella graminicola* (Fuckel) Schroter, the strength of the correlation between pairs of DMIs has changed over time (Gisi, U., 1997 pers. comm.). Similarly, closely related pyrethroid insecticides differ in their susceptibility to metabolic resistance mechanisms[54] and to *super-kdr*.[55] However, the differences have rarely been exploited in formal resistance management strategies.

3.1 Strategies

To be most effective, management strategies should always begin at the very start of the lifespan of a new pesticide and not in response to a perceived problem. The adage 'prevention is better than cure' applies fundamentally to resistance management. However, having carried out a vast array of studies, little more can be done than to estimate whether the overall resistance risk is high (e.g. benzimidazoles), low (e.g. multi-site inhibitors such as dithiocarbamates), or somewhat intermediate, as seems likely for most new fungicidal inhibitors. Based on the information available in the public domain on phenylpyrroles and quinoxyfen, and from Zeneca's research with strobilurins, this seems a reasonable conclusion. At this point, initial strategies should be conservative and measures put in place to monitor the effectiveness of the strategies, particularly in situations perceived to represent higher risks. This might include crops with many spray applications per season or where grower practices are more difficult to control—the diverse arena of horticulture is a good example.

Whatever the risk, all strategies are variations on a theme of reducing selection pressure on any one resistance mechanism in the target pest population. Standard approaches to lower selection pressure include limiting the number of applications, exploitation of refugia, alternation or mixture with pesticides from different cross-resistance groups, and directing the product against the most vulnerable life-stage of the target. These and other considerations must be balanced against the perceived resistance risk and the likelihood of growers adopting recommendations. Indeed, none of these measures is unduly complex or associated with a high degree of risk. Most could be recommended prior to the launch of a new product as a 'best bet' strategy.[56] However, it is arguably not commercially astute for a company to engage in unilateral pro-active resistance management practices. This is discussed in further detail in Wege,[57] and is a very strong argument for industry bodies such as IRAC and FRAC.

3.2 Implementation of strategy

The implementation of management practices is by far the most challenging part of the process in successful resistance management.[57] A good example of this difficulty may be found in an analysis of the newly introduced insecticide, imidacloprid. The manufacturers were diligent in conducting baseline monitoring and making appropriate resistance management recommendations for the product,[58] yet there has been a widespread disregard of resistance management by end-users in many markets where this product is sold.

There are ways in which the agrochemical companies can assist in the successful implementation of resistance management. For example, one of the first steps in successful resistance management is to identify the pest target accurately. With insect pests, this can be achieved using relatively sophisticated technology such as the Lepton ELISA kit[59] which is used in the field to distinguish eggs of the susceptible *Helicoverpa punctigera* Wallengren from pyrethroid-resistant *Helicoverpa armigera* Hübner in Australian cotton. Similarly, accurate

identification is important in aphid control, where precise distinction of species with a known history of resistance problems such as *M. persicae* Sulz, *A. nasturtii* Kltb and *Nasonovia ribis nigri* (Mosley) from other, more readily controlled species, can strongly influence product choice (or method of control) and whether or not effective control is achieved both in the immediate and longer term. Effective determination of the above aphid species can be achieved in the field using a $\times 10$ hand-lens and a good understanding of aphid biology and host range. To this end, Zeneca Agrochemicals has been running courses in aphid identification for major distributors and grower groups in order to facilitate informed decision-making for aphid control in arable crops.

Precise resistance management recommendations have been included on product labels, most notably on acaricides.[57] However, Clarke *et al.*[60] rightly point out that many labels have non-specific resistance management statements which are not constructive in the process of changing practices. There are some obvious practical reasons why generic statements have been used, most particularly on broad-spectrum products where it is impracticable to put specific guidelines for each of the 50 or so crops listed on the label. It will be on narrow-spectrum products which are used in relatively sophisticated cropping systems that this means of communicating resistance management guidelines will be most effective, that is, provided it is accompanied by other means of communication.[57] Label statements will only be effective if statements are consistent across the products in a particular market and are supported by all factions of the industry. This can only be achieved through groups such as IRAC and FRAC.

However, in some cases the chain from the resistance specialist to the grower can be a long one, and messages are easily distorted. This at least indicates that the basic information generated by industry must be clear and consistent. At the individual company level this means sound technical information widely available; clear recommendations as part of the chemical label or in accompanying literature and an openness to discuss resistance data and issues at all levels. At an industry level the various Resistance Action Committees can help align the companies with a consistent stance. There have been successes, and the continuing efficacy of the SBI fungicides and the pyrethroids is due in no small part to the efforts of various FRAC and IRAC working groups to generate data, agree strategic principles and to search out the key influencers to drive these principles into practice.

4 MONITORING AFTER COMMERCIALISATION

Baseline monitoring of target susceptibility is increasingly conducted by agrochemical companies in the development phase of a product. These data then provide a means of determining the onset of resistance and dealing with grower complaints. A good example of this practice may be found in Elbert *et al.*[58] Once a product has been launched, the financial and manpower burdens of wide-scale monitoring often strongly orientate the process towards the provision of customer service and complaint investigation. However, the degree to which this occurs is dependent on the spectrum of activity of the compound, the length of time since launch, its profit margin and the degree of investment/ownership of the product.

The specific aphicide, pirimicarb, was launched at a time when resistance monitoring was in its infancy. However, the value of the product to both the producer and the customer was realised early in its development, and prompted commercial investment in monitoring.[61] Since then, Zeneca has continually invested in monitoring the susceptibility of aphids, both as a means of determining when resistance occurs and also as a means of providing valuable data which assist in the promotion of the product, which is effective against most common resistant strains.

The recent appearance of modified acetylcholinesterase resistance[62] in UK populations of *M. persicae* has prompted a further expansion of monitoring by the producer of one of the two products most affected by this resistance mechanism. This monitoring enables the company to understand fully the extent and frequency of this and other resistances in this species and to make the appropriate recommendations to its sales force and customers.

Nonetheless, any initial resistance management strategy can only be an estimate of what is optimal. Strategies need adjustment in response to external changes. New pesticides arrive, agronomic practice changes, the evolution of resistance is a dynamic which the industry must observe and to which it must respond. The problem is a fundamental one, be it for fungicides, herbicides or insecticides. How do you detect resistance at an early stage when resistant individuals may be at a low frequency (but increasing) in the population? Experience shows that current approaches to monitoring rarely detect resistance at frequencies less than 0·5%. The recent detection of anilinopyrimidine-resistant strains of *B. cinerea* taken from trial plots at a frequency of 0·4%[49] underlines the point. This was a major long-term study involving considerable resources. Indications even earlier than this would be advantageous and the agrochemical industry must find ways of screening larger samples more quickly. Brent *et al.*[63] showed that, to detect a resistance frequency of 0·1% with 95% confidence, approximately 3000 isolates need to be assayed. Developments already made to accommodate high-throughput chemical screens for chemical invention need to be adapted to high-throughput target screens for resistance detection. If detection levels of 0·01% or

less could be achieved, strategies could be adjusted more judiciously.

However, with the DMI fungicides it has been possible to observe relatively small changes in resistance with small sample sizes (several hundreds), due to the quantitative nature of the resistance mechanism.[64] In this case, continued monitoring has provided a valuable insight into the relative success of anti-resistance strategies and the SBIs continue to be major fungicide players after over 20 years of widespread use.

5 CASE STUDY: MANAGEMENT OF RESISTANCE TO LAMBDA-CYHALOTHRIN IN ASIAN COTTON MARKETS

It is valuable to analyse a market situation where anti-resistance recommendations have been married to the realities of commercial spray programmes, in order to assess the success of the strategy and its communication, and the need for continued monitoring to allow necessary adjustments to be made. A suitable case study to examine is the management of resistance to pyrethroids, including lambda-cyhalothrin (sold under the trade marks Karate and Kung Fu) in cotton in Asia. As stated earlier, the ideal approach to practical resistance management involves:

(i) understanding the physical, biochemical and behavioural mechanisms by which major pest species survive a normally lethal dose of each product group used by growers,
(ii) devising a feasible strategy which reduces the genetic selection of any one mechanism to the point where product 'failure' is avoided,
(iii) implementing and sustaining specific tactics for crop protection on all farms in a locality, primarily through education of end-users and suppliers, and
(iv) monitoring pests so that trends in resistance frequency can be quantified and tactics modified as necessary year-on-year.

However, in a situation like Asian cotton, success in restricting the spread of resistance must be achieved from less ideal approaches, for example where little is known about the biochemical changes in insect populations or the seasonal movements of resistant strains. Ultimately any useful strategy amounts to preventing over-use of any one group of products as a precautionary measure, and sophisticated research on altered sites and enzyme systems or monitoring systems are rarely essential before relevant action is possible.

The key to effective implementation of resistance management lies in the co-operation of all sectors (public and private) concerned with crop protection, good communication of best practices, and the commitment of growers and advisers in the knowledge that current pesticide products are a precious resource vital to profitable production. Such co-operation and commitment has been seen to varying degrees, most notably in the cotton-growing areas of Australia, and to a lesser extent in USA cotton, as well as in many fruit-growing areas of Europe and North America. This is largely related to discipline and technical support at end-user level and the ability of educated growers to understand and adopt concepts of IPM.

In the developing economies of India, Pakistan and China, where millions of small-holders grow cotton and vegetables in a mosaic of cropping patterns, an altogether different challenge in resistance management is presented. The opportunity to co-ordinate a system in which hundreds of generic and branded pesticides are sold through private dealers to mostly untrained farmers of limited literacy is severely limited and places great demands not only on improved training and better-resourced extension services, but also on the multi-national companies to provide adequate technical support for their products. This needs to include the promotion of IRM (insecticide resistance management) and IPM in realistic ways, rather than the pursuit of maximum sales whatever the size of infestations and species of pests occurring from year to year. Through the international manufacturers' organisation, GCPF (Global Crop Protection Federation), the Agrochemical Industry is increasing its efforts to improve customers' knowledge and skills in using pesticides within an IPM framework. The task in developing countries is daunting, nowhere more so than in cotton crops known to receive up to 40 chemical sprays in one season. To tackle IRM and IPM on a meaningful scale, more dialogue and joint project funding is needed between the leading manufacturers, GCPF, Government agencies and non-governmental agencies such as FAO and World Bank, and it is encouraging that, despite some of the deep-rooted suspicion of motives in the agrochemical industry, this is beginning to happen (Seth, A., 1997, pers. comm.)

A number of factors can constrain the adoption of IRM in a country like India[65] (Table 1). The relative cheapness, especially of generic products, and the encouragement of tank mixes to overcome problems of reduced efficacy have essentially led to a crisis in crop protection in southern India, with levels of resistance in populations of the American bollworm, *H. armigera*, so high in most years that the economic viability of cotton and pulse production is threatened, and switching to less affected crops is practised. The same can be said for the Yellow River basin in China, where the 'knee-jerk' reaction of farmers to poor control is to apply two- and three-way mixtures of products at ever shorter intervals in order to protect their livelihoods. Some of these mixtures have been shown to overcome pyrethroid resist-

TABLE 1
Constraints to the Adoption of IRM in India

• Lack of community action amongst farmers
• Poor links between research scientists and extension officers
• Lack of resources and support for extension officers
• Lack of effective training in pest control for farmers
• Poor application techniques (failure to spray at correct time)
• Past conditioning of farmers to adopt calendar-based pesticide applications
• Profit-oriented attitude of dealers and distributors
• Dependence of many farmers on credit from dealers
• Cheapness of insecticides (encouraging over-use)
• Tank-mixing of several products to overcome reduced efficacy
• Variable crop planting dates (succession of host plants for pests)
• Use of long-maturing crop varieties (allows more pest generations to develop)

ance in the short term, but resistance will inevitably develop to all mixture components, partly because under-dosing is commonplace.

When Zeneca launched its new-generation pyrethroid insecticide, lambda-cyhalothrin, in Indian cotton in 1996, it was recognised that its efficacy against bollworms would only be optimised by applications at the right time, the correct rate, and when alternated with other insecticides possessing a different mode of action. Too many cotton farmers use the same product too often in one season and wait until an infestation is firmly established and visibly damaging the crop before starting to spray. Training of internal technical and marketing staff, dealers and eventually growers was seen as critical to success, and to this end programmes in the safe and effective use of insecticides began in the Guntur region of Andhra Pradesh in 1997 (Guest, P. J., 1997, pers. comm.). Participatory training in the identification of eggs and young larvae of various pest species, the use of peg boards to monitor population development, and the selection of nozzles and calibration of hand-held sprayers to ensure accurate dosing, have all featured strongly in these programmes. Even when resistance levels due to oxidative metabolism are quite high, the recommended dose applied to hatching eggs and early-instar larvae still delivers cost-effective control, and this is the message being promoted not only in India, but in China, Pakistan and other cotton-growing countries.

IRAC India is also active in encouraging more rational use of pesticides. Field demonstrations of product rotations based on different modes of action, in comparison to farmers' own practices, are held in various states, and a recommended approach to bollworm control and to resistance management is widely distributed through leaflets and posters (Table 2)

TABLE 2
IRAC India's Recommendations for Control of *Helicoverpa armigera* in Cotton

• Timely control of eggs and young larvae at an early crop stage to prevent excessive population build-up and crop damage
• Recommended doses of products to be used either alone or in mixtures
• Good spray coverage of the upper surfaces of leaves and buds where bollworm moths generally lay their eggs
• Careful monitoring of crops and protection of the early flush of squares and bolls to ensure high yields and more profitable returns
• Maintenance of sprayers in good working order and choice of an appropriate nozzle
• Use of correct volumes of water for the growth stage of the crop
• Alternation of different groups of bollworm-approved insecticides (pyrethroids, organophosphates, carbamates, endosulfan etc.) throughout the season to reduce selection pressure from any one group.

(Murthy, K. S., 1997, pers. comm.). The overall aim is to convince small-holders of the advantages of IRM and IPM in the longer term, and for other components of IPM such as resistant varieties (including Bt varieties) and biopesticides to be made available and economically attractive. Providing a wider range of cost-effective and easy-to-use crop protection tools is one of the best ways to reduce selection pressure on genes for pyrethroid resistance.

The ultimate measure of management success is that resistance levels in field populations are maintained such that cost-effective pest control continues. In the cotton-growing regions of Asia, resistance in bollworms is undeniably increasing over time[66] (Fig. 1), but there is little or no research relating the recorded

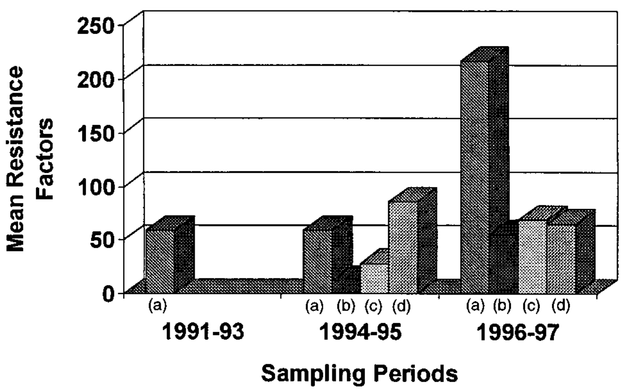

Fig. 1. Changes in resistance factors of *Helicoverpa armigera* to various pyrethroid insecticides in Pakistan (M. Ahmad, 1997, pers. comm.) (a) Cypermethrin, (b) lambda-cyhalothrin, (c) deltamethrin, (d) cyfluthrin.

resistance factors to the field performance of the recommended doses of particular products. Field failure is much more associated with incorrect timing and dosing, poor application techniques and adulterated generic products. The contribution of resistance *per se* needs more scientific investigation, although, as stated earlier, lack of such data should not prevent or delay the implementation of a management strategy.

6 THE FUTURE

Many of the problems with resistance management stem from a lack of knowledge of the life processes being influenced, such as basic biochemical, genetic and even epidemiological information about the target organisms, and, too often, 'model' targets are used to glean information.

For example, for fungicide research, mutants isolated in the laboratory often have not appeared in the field, and apparent laboratory indications of reduced fitness have quickly been confounded by field populations. To make progress, it is essential to study target pathogens at the biochemical and genetic level to begin to understand these basic phenomena. Molecular diagnostic techniques are progressing rapidly in the medical area and these must be adapted to help in studying resistance-gene frequencies rather than just the frequency of resistance phenotypes. Problems of sampling and high-throughput testing must be addressed if such studies are to be anything more than retrospective. An intimate knowledge of target sites and resistance mechanisms is required to apply this approach, though, as the discovery process becomes increasingly rational, the prospects for this are good. An improved knowledge of resistance-gene frequencies combined with a better understanding of the genetic structure of populations should help to revitalise the mathematical models which so far have had only limited impact on resistance management.

Even if better strategies can be developed through improving the science base, the challenge of education and communication must be tackled comprehensively in order to steer the desired course. This entails:

(a) The provision of field diagnostic kits, (i) to enable rapid identification of compound-specific resistance and (ii) to determine the mechanism of resistance present to allow an effective substitute to be used.
(b) The introduction of an effective product labelling system, as for Australian herbicides, which even illiterate farmers can follow in order to rotate modes of action.
(c) Ensuring that proper and workable resistance management guidelines are in place at the time of new product launch.
(d) Industry increasing its effort to train end-users and dealers in IRM and IPM.
(e) Ensuring that improved technology is adopted to apply pesticide at the right time and in the right place (GPS, lure-and-kill, preventative spray timings, seed treatments etc) so that selection pressure is lowered.
(f) The adoption of properly designed IPM programmes which avoid over-use of any one technique.

None of the above is trivial. Success is dependent on putting in place fundamental research in both the industrial and academic arenas, and the infrastructure to communicate with farmers and growers. The prize is the long-term protection of the active ingredients that will contribute to the demanding food and fibre production targets of the next millennium.

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